Reactions in the Annual *Medicago* spp. Core Germ Plasm Collection to *Phoma medicaginis*

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ABSTRACT

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The annual *Medicago* spp. core collection, consisting of 201 accessions, represents the genetic diversity inherent in 3,159 accessions from 36 annual *Medicago* spp. This germ plasm was evaluated for resistance to spring black stem and leaf spot caused by *Phoma medicaginis*. Spring black stem and leaf spot is a major destructive disease in perennial alfalfa (*Medicago sativa*) grown in North America, Europe, and other temperate regions. Disease control is based principally on the use of cultivars with moderate levels of resistance. Evaluation of the core collection was conducted using standardized environmental conditions in growth chambers, and included the *M. sativa* standard reference cultivars Ramsey (resistant) and Ranger (susceptible). The degree of resistance found among accessions within species was variable, but most annual species and accessions were susceptible. Most accessions from 10 species exhibited high disease resistance. These included accessions of *M. constricta, M. doliata, M. heyniana, M. laciniata, M. lesinsii, M. murex, M. orbicularis, M. praecox, M. soleirolii,* and *M. tenoreana.* Most of the accessions within *M. arabica, M. minima, M. lanigera, M. rotata, M. rugosa, M. sauvagei,* and *M. scutellata* were highly susceptible. Disease reactions among some accessions within species were highly variable. On a 0-to-5 disease severity scale, ratings ranged from 0.67 (PI 566873) to 4.29 (PI 566883) within accessions of *M. polymorpha*. Most of the *M. truncatula* accessions were susceptible, with a mean of 3.74. Resistant reactions were similar to those found in incompatible interactions with *P. medicaginis* and alfalfa, which have been associated with specific genes leading to the production of isoflavonoid phytoalexins. The large genetic variability in annual *Medicago* spp. offers potential for locating and utilizing disease resistance genes through breeding or genetic engineering that will enhance the utilization of *Medicago* spp. as a forage crop.

Additional keywords: alfalfa, disease resistance, lucerne

Recent interest in the use of annual species of *Medicago* as a cover and forage crop for use in sustainable agriculture systems in the United States has prompted both an evaluation of the diversity of germ plasm that exists and the development of an annual *Medicago* spp. (medics) core collection. This collection represents the genetic diversity inherent in 3,159 accessions from 36 species of annual *Medicago* contained in the United States National Plant Germplasm System (10,12). A subset of 1,240 accessions was evaluated for morphological and agronomic traits, and accessions were chosen within a species to represent the greatest diversity in geographical origin (11). The selected core collection of 201 accessions from 33 species was evaluated at seven locations across the United States, and found to remain stable across environments and to represent the variability of the germ plasm collection (11). Annual medics have potential use in North America as weed-suppressing smother crops, cover crops in row crop production, and for short-season forage crops (5,15).

Annual *Medicago* spp. are native to regions surrounding the Mediterranean Sea, and commercial cultivars are important forage crops in Australia and South Africa. The species that are most widely grown and from which commercial cultivars have been developed are *M. littoralis, M. murex, M. polymorpha, M. rugosa, M. scutellata,* and *M. truncatula* (5). Foliar diseases frequently occur in these species and cause significant yield losses (1–3,24). The same foliar diseases cause significant losses in forage and seed yield in *M. sativa* (30,32,34). The availability of disease-resistant accessions in the annual medic collection will enhance the utilization of these species in North American agro-ecosystems, and also may serve as a potential source of new or novel resistance genes amenable for incorporation into adapted genotypes.

Sources of resistance in annual *Medicago* spp. to one of the most important indigenous foliar diseases of alfalfa, anthracnose caused by *Colletotrichum trifolii* Bain & Essary, have been reported (23,26).

Table 1. Virulence of *Phoma medicaginis* isolates to *Medicago sativa* cultivar Ranger

P. medicaginis isolate	Origin	Host	Disease severity[a]
P198	Canada	*M. sativa*	2.4
P298	Aichi, Japan	*M. lupulina*	3.6
P398	Montpellier, France	*M. truncatula*	2.7
P598	Sweden	*M. sativa*	3.9
P798	Battle Ground, IN	*M. sativa*	4.7
P1598 (T-430)	Tucson, AZ	*M. sativa*	4.1
P1698 (T-432)	Tucson, AZ	*M. sativa*	3.9
P1798 (T-431)	Tucson, AZ	*M. sativa*	4.0
P1998 (5D85-8)	Pullman, WA	Unknown	3.4
1585	State College, PA	*M. sativa*	4.5
647	State College, PA	*M. sativa*	3.6
979	Bucks Co. PA	*M. sativa*	4.7
866	Wisconsin	*M. sativa*	4.7
1065	State College, PA	*M. sativa*	4.0
W13	West Madison, WI	*M. sativa*	2.6
W2	West Madison, WI	*M. sativa*	3.6
WA2	Wisconsin	*M. sativa*	3.2
PACRS1	Wisconsin	*M. sativa*	4.4
PA2453	Wisconsin	*M. sativa*	3.5
LSD[b]	...	...	0.7

[a] Data represents means from six pots from two experiments that were combined for analysis. Plants were evaluated on a 0-to-5 scale, where 0 = healthy, symptom-free top growth; 1 = small (<2 mm), brown or black lesions or flecks; no defoliation; 2 = larger (2 to 3 mm), discrete lesions; lesions may be on leaves and petioles; usually no chlorosis or defoliation; 3 = large (>3 mm) lesions; no defoliation; petiole lesions; 4 = lesions >3 mm; chlorosis, dead leaves, or defoliation; and 5 = most of the leaves necrotic or defoliated; dead plant.

[b] Least significant difference (LSD) was calculated according to Fisher's protected test at $P \leq 0.05$.

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DeHaan et al. (9) were the first to report the existence of Phytophthora root rot on annual medics, and they found that most cultivars and accessions within eight annual medic species were susceptible. Recent evaluations of reactions in this collection to powdery mildew caused by *Erysiphe pisi* DC. and downy mildew caused by *Peronospora trifoliorum* de Bary revealed a wide range of disease reactions (35,36). Economically important diseases for which evaluations have not been made include spring black stem and leaf spot caused by *Phoma medicaginis* (Malbr. & Roum.) var. *medicaginis* Boerema (syn. *P. herbarum* Westend. var. *medicaginis* Fckl. and *Ascochyta imperfecta* Peck), and Leptosphaerulina leaf spot caused by *Leptosphaerulina briosiana* (Pollacci) J. H. Graham & Luttrell (1,2,23,29,32).

Spring black stem and leaf spot caused by *P. medicaginis* is a common and often serious disease in North America and Europe. It is characterized by premature leaf drop; stem, leaf, and petiole necrosis; and crown and root rot of mature plants (32). Losses in yield and quality can be high, and cultivars with acceptable levels of resistance are not available (28). In a survey of foliage diseases on 42 accessions of annual *Medicago* spp. in South Africa, spring black stem and leaf spot was the most common of nine diseases recorded on aboveground plant parts (24). Disease control is based principally on the use of a few moderately resistant cultivars, early cutting to reduce leaf loss, and fungicides (32,34). Another species of *Phoma*, *P. sclerotioides*, the cause of brown root rot, has recently been reported on alfalfa in the United States (21).

Defense reactions to *P. medicaginis* have been associated with the production of pterocarpan and isoflavonoid phytoalexins following infection (13,19,27). Fungitoxic phenolic compounds have been identified from several annual *Medicago* spp., but their role in disease resistance has not been investigated (22). Annual *Medicago* spp. exhibiting disease resistance may contain novel disease resistance genes or produce fungitoxic secondary metabolites that could be exploited for use in adapted perennial alfalfa via genetic engineering. Modifying flavonoids has been shown to increase disease resistance in alfalfa (18,20,27).

Alfalfa is an autotetraploid that suffers from inbreeding depression, resulting in self-sterile accessions after a few generations of self-pollination. Cultivars are very heterogeneous and usually are developed as synthetics. Because most annual medics are diploids ($2n$ = 14 or 16), autogamous, and self-fertile, these species are genetically simpler to study and easier to manipulate than alfalfa. Some species, such as *M. truncatula*, are transformable (7,33) and are being used as a model legume system (6,8), to study symbiosis-related plant

Table 2. Evaluation of the annual *Medicago* core collection for resistance to spring black stem and leaf spot caused by *Phoma medicaginis*

Medicago spp. and PI number[a]	Country of origin	Disease severity[b]
arabica		
PI 495200	France	4.00
PI 495212	Hungary	4.00
Mean		4.00
blancheana		
PI 495215	Unknown	1.33
PI 495216	Turkey	2.33
PI 495222	Lebanon	1.33
PI 495223	Lebanon	2.33
PI 495227	Hungary	2.33
PI 505415	Spain	1.83
PI 505416	Spain	2.33
Mean		1.97
ciliaris		
PI 368928	USA (California)	2.67
PI 442645	Turkey	2.17
PI 498731	Czechoslovakia	2.17
PI 498750	Lebanon	2.17
PI 498784	Tunisia	2.50
PI 498785	Morocco	2.67
Mean		2.39
constricta		
PI 495240	Greece	1.71
PI 534177	Bulgaria	2.00
PI 534182	Cyprus	1.83
Mean		1.85
coronata		
PI 498790	Greece	2.80
PI 498805	Lebanon	1.50
Mean		2.15
disciformis		
PI 487317	Bulgaria	2.10
PI 487321	Greece	2.85
PI 487322	Italy	3.40
PI 487333	Cyprus	2.40
Mean		2.69
doliata		
PI 495278	Lebanon	2.30
PI 505420	Spain	1.50
Mean		1.90
doliata var. *muricata*		
PI 534202	Lebanon	1.67
PI 534211	Algeria	1.50
Mean		1.59
granadensis		
PI 498810	Israel	3.17
PI 498812	Turkey	2.22
PI 498813	Turkey	1.66
PI 498817	Turkey	2.83
Mean		2.47
heyniana		
PI 537136	Greece	1.86
intertexta		
PI 498824	Portugal	2.42
PI 498828	Italy	1.75
PI 516649	Morocco	2.33
PI 516650	Morocco	2.43
PI 535606	Tunisia	2.67
PI 535607	Tunisia	2.17
Mean		2.30

(continued on next page)

[a] Six-digit numbers are plant introduction (PI) numbers used by the National Plant Germplasm System of the United States Department of Agriculture.

[b] Plants were evaluated for disease severity on a 0-to-5 scale. Data represent the mean of ratings from at least six replicate pots of 10 plants each from two experiments. Plants in each pot were rated on the basis of the lesion type and amount of foliar and stem necrosis and chlorosis, where 0 = healthy, symptom-free top growth; 1 = small (<2 mm), brown or black lesions or flecks; no defoliation; 2 = larger (2 to 3 mm), discrete lesions; lesions may be on leaves and petioles; usually no chlorosis or defoliation; 3 = large (>3 mm) lesions; no defoliation; petiole lesions; 4 = lesions >3 mm, chlorosis, dead leaves or defoliation; and 5 = most of the leaves necrotic or defoliated; dead plant. Least significant difference (1.3) was calculated according to Fisher's protected test at $P \leq 0.05$.

[c] Standard reference cultivars for spring black stem and leaf spot resistance in *M sativa*.

Table 2. *(continued from preceding page)*

Medicago spp. and PI number[a]	Country of origin	Disease severity[b]
italica		
PI 384640	Morocco	3.33
PI 385014	Tunisia	3.00
PI 459188	Turkey	1.67
PI 566864	Spain	1.67
PI 566865	USA Georgia	1.33
PI 566866	Italy	1.33
PI 566867	Morocco	1.33
PI 566868	Sweden	1.00
Mean		1.83
laciniata		
PI 498839	U.K.	0.00
PI 498841	Israel	1.22
PI 498842	Czechoslovakia	0.00
PI 498853	Spain	2.00
PI 498864	Spain	1.67
PI 498890	Spain	2.00
PI 498916	Morocco	0.00
PI 498918	Iraq	3.00
PI 535738	Libya	2.00
Mean		1.32
lanigera		
PI 498930	Former USSR	4.33
lesinsii		
PI 534233	Israel	1.67
PI 537259	Australia	1.14
Mean		1.41
littoralis		
PI 385006	Tunisia	1.83
PI 517206	Australia	1.50
PI 537168	Cyprus	0.33
PI 537171	Lebanon	1.50
PI 537201	Italy	0.50
PI 537207	Spain	2.67
PI 537222	Morocco	0.00
Mean		1.19
lupulina		
PI 189128	Denmark	1.00
PI 202038	Argentina	0.00
PI 215245	USA Nebraska	2.00
PI 227452	Iran	0.00
PI 234821	Switzerland	0.00
PI 251834	Italy	0.83
PI 269926	Pakistan	2.33
PI 290723	U.K.	0.33
PI 304527	Turkey	1.50
PI 308059	Czechoslovakia	1.50
PI 314538	Former USSR	2.00
PI 319026	Spain	1.50
PI 452459	Canada	0.67
PI 532942	Nepal	3.33
PI 566869	Netherlands	1.50
Mean		1.23
minima		
PI 227032	Iran	2.57
PI 499022	Lebanon	2.14
PI 499072	Italy	1.86
PI 499080	Turkey	1.83
Mean		2.10
murex		
PI 308062	Czechoslovakia	1.50
PI 495350	Italy	0.83
PI 495379	France	1.00
PI 534231	Canada	1.00
PI 516720	Morocco	1.17
Mean		1.10
muricoleptis		
PI 495401	Italy	2.29
noeana		
PI 495407	Turkey	3.75
PI 495414	Unknown	3.71
Mean		3.73

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genes (4), and to manipulate and enhance resistance to foliar pathogens based on inducible or altered levels of phytoalexins (4,13,17).

There is a need to evaluate populations of annual *Medicago* spp. for resistance to destructive fungal diseases and to determine whether or not defense expression resistance is based on the production of new or altered levels of fungitoxic flavonoids and isoflavonoids. The objective of the present study was to evaluate resistance to spring black stem and leaf spot in the annual *Medicago* spp. core collection, and to determine differences in virulence among *P. medicaginis* isolates to a susceptible line of *M. sativa*.

MATERIALS AND METHODS

Although *P. medicaginis* is not classified by race, isolates obtained from seed vary significantly in virulence to excised *M. sativa* foliage (16). Selection of an isolate for use in screening the annual medic collection, therefore, was based on the ability of the isolate to produce severe disease symptoms in alfalfa. To determine variation in virulence among a collection of diverse isolates and to enable selection of a highly virulent isolate for evaluation of the annual medics, symptom expression of isolates was evaluated on the *M. sativa* susceptible cultivar Ranger (26,30). Inoculum preparation, plant growth conditions, and disease evaluations are as described below for evaluations of the annual *Medicago* spp. core collection. The virulence of isolates was determined in 10 plants per pot using three pots per isolate, and the experiment was repeated. Data were combined for analysis.

Plants of 200 accessions from 33 species in the annual *Medicago* spp. core collection were evaluated in growth chambers for resistance to spring black stem and leaf spot caused by *P. medicaginis*. *M. sativa* cvs. Ramsey (moderately resistant) and Ranger (susceptible) were included as standard reference cultivars (30). Plant growth conditions, inoculation, and evaluation of accessions for spring black stem and leaf spot resistance were conducted by a modification of standardized procedures recommended for evaluating this disease in alfalfa (30,32). Seed of each accession were scarified by abrasion against sandpaper and planted in 10-cm pots of a 50% mixture of pasteurized field soil and Jiffy-Mix Plus (Jiffy Products of America Inc., Batavia, IL) potting mix at a rate of approximately 15 seed per pot. Plants were placed in a growth chamber at 23°C with a 16-h fluorescent light photoperiod for 14 days. The number of seedlings per pot was determined 10 days after planting. Those with 10 or more seedlings per pot were inoculated when growth was sufficient for evaluation, generally 2 to 3 weeks after seeding, depending on the species. Plants were spray inoculated to just prior to run-off (approximately 2 ml

per pot) with a spore suspension of *P. medicaginis* isolate T-431. Pots were incubated in a mist chamber at 23°C for 48 h, then returned to a growth chamber for disease development. The relative humidity in the chamber was maintained at 60%, and plants were evaluated for disease reaction 8 to 10 days after inoculation.

Inoculum was prepared by suspending spores of *P. medicaginis* isolate T-431 in sterile distilled water containing Tween 20 at two drops per liter. The inoculum concentration was adjusted to 3 to 4 × 10^5 spores/ml. The spore density is less than that which has been used in standard tests (1 to 4 × 10^6) because preliminary experiments demonstrated that the lower concentration was sufficient to produce high levels of disease. Spores were obtained from 7-day-old cultures grown on half-strength oatmeal agar (36 g of Difco oatmeal agar, 1 liter of distilled water, 7.5 g of agar) at 21°C under 12 h of fluorescent light.

A minimum of 10 seedlings per pot with three replications per accession were evaluated. Some of the accessions exhibited poor germination; therefore, additional sets of pots of these accessions were planted, evaluated, and combined for analysis in the reported experiment. Due to space limitations for inoculation and plant growth chambers, approximately 50 accessions, including the standard cvs. Ramsey and Ranger, were evaluated per run. The experiment was repeated after all lines had been evaluated from the first experiment. The data from at least six pots of 10 plants per pot from the two experiments were combined for analysis. Means for the germ plasm entries were not adjusted based on the disease severity on the standard cultivars.

Disease reactions in the annual medic accessions were evaluated based on class descriptions using a 0-to-5 scale, designed to account for the lesion size and the amount of defoliation, petiole blight, chlorosis, and necrosis. Gray et al. (16) reported that, in *M. sativa*, ratings for disease should include these symptoms because each appears to play an equally important role in pathogenesis. The rating scale is slightly modified from that described by Salter and Leath (30) for evaluating *M. sativa* resistance to spring black stem and leaf spot. In preliminary experiments, we found that we could distinguish an additional rating class among the annual medic species and take into account additional symptom details. Thus, we deviated from the rating system of Salter and Leath (ratings of 1 to 5) and constructed a rating system to include an additional class (0 to 5). The plants in each pot were rated as follows: 0 = healthy, symptom-free top growth; 1 = small (<2 mm), brown or black lesions or flecks; no defoliation; 2 = larger (2 to 3 mm), discrete lesions; lesions may be on leaves and petioles; usually no chlorosis or defoliation; 3 = large (>3 mm) lesions; no defoliation; petiole lesions; 4 = lesions >3 mm, fruiting; chlorosis, dead leaves, or defoliation; 5 = most of the leaves necrotic or defoliated; dead plant. According to Salter and Leath (30) alfalfa plants with scores of 0 to 2 are considered resistant. The expected percentage of alfalfa plants classified as resistant within standard check cvs. Ramsey (moderately

Table 2. *(continued from preceding page)*

Medicago spp. and PI number[a]	Country of origin	Disease severity[b]
orbicularis		
PI 210425	Greece	1.17
PI 251474	Turkey	1.17
PI 283645	Morocco	1.50
PI 292421	Israel	2.17
PI 505425	Spain	1.17
PI 566870	Romania	1.00
PI 566871	Italy	2.00
PI 566872	Italy	1.67
Mean		1.48
polymorpha		
PI 478531	Peru	1.67
PI 493292	Portugal	2.17
PI 493293	Portugal	0.83
PI 566873	Italy	0.67
PI 566874	Greece	1.33
PI 566875	Cyprus	1.17
PI 566876	USA California	3.67
PI 566877	Italy	1.33
PI 566878	USA Georgia	3.43
PI 566879	Germany	2.83
PI 566880	Belgium	1.33
PI 566881	Lebanon	4.14
PI 566882	Greece	4.00
PI 566883	France	4.29
PI 566884	Syria	4.14
PI 566885	Morocco	3.86
Mean		2.55
polymorpha var. *brevispina*		
PI 186329	Australia	3.79
PI 197340	Australia	3.00
PI 197530	Algeria	2.50
PI 226648	Iran	3.33
PI 368949	Chile	3.33
PI 385017	Tunisia	2.00
Mean		2.99
polymorpha var. *polymorpha*		
PI 206695	Turkey	2.50
PI 244312	Spain	2.33
PI 250782	Afghanistan	2.33
PI 253448	Yugoslavia	4.00
PI 283657	Sweden	2.00
PI 286534	Ethiopia	2.50
PI 292427	Israel	1.67
PI 302926	Spain	1.67
PI 308055	Czechoslovakia	1.50
PI 319036	Spain	2.33
PI 404795	Uruguay	3.33
PI 459130	Turkey	2.50
Mean		2.39
praecox		
PI 495429	Greece	1.50
PI 495434	France	1.67
Mean		1.59
radiata		
PI 340800	Hungary	3.25
PI 459142	Turkey	3.00
PI 459145	Turkey	2.25
PI 459146	Turkey	2.81
Mean		2.83
rigidula		
PI 230350	Iran	1.50
PI 233250	Israel	1.33
PI 319048	Spain	3.75
PI 495517	Greece	3.00
PI 534236	Turkey	3.25
PI 534250	Turkey	4.00
Mean		2.81

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resistant) and Ranger (susceptible) is 25 and 3%, respectively (30).

The disease reaction data was subjected to analysis of variance with the SAS general linear models procedure (SAS release 6.11; SAS Institute, Cary, NC). Means were compared using Fisher's protected least significant difference at $P \leq 0.05$.

Table 2. *(continued from preceding page)*

Medicago spp. and PI number[a]	Country of origin	Disease severity[b]
rotata		
PI 292430	Israel	4.33
PI 495576	Unknown	4.33
PI 495577	Italy	4.75
PI 495583	Turkey	4.75
PI 495586	Lebanon	3.75
PI 537236	Czechoslovakia	4.33
Mean		4.37
rugosa		
PI 308061	Czechoslovakia	4.00
PI 368962	Greece	5.00
PI 442893	Australia	3.75
PI 487363	Portugal	3.66
PI 487374	Lebanon	4.00
PI 487377	Spain	4.00
PI 487379	Italy	3.75
PI 487382	Italy	3.66
PI 487386	Tunisia	4.33
PI 535534	Tunisia	4.00
PI 535537	Tunisia	3.66
Mean		3.98
sauvagei		
PI 499152	Morocco	5.00
PI 499153	Unknown	4.80
Mean		4.90
scutellata		
PI 161415	Uruguay	3.60
PI 197806	Australia	4.00
PI 197817	USA	4.20
PI 197821	Cyprus	4.50
PI 292432	Israel	4.00
PI 487392	Sweden	4.50
PI 487393	Cyprus	2.80
PI 487394	Germany	4.20
PI 487396	Hungary	4.20
PI 487403	Turkey	3.75
PI 487409	Lebanon	4.00
PI 487411	Italy	4.20
PI 516907	Morocco	4.40
PI 517255	Ethiopia	4.33
PI 535643	Tunisia	3.60
PI 535644	Tunisia	4.50
PI 535645	Tunisia	2.80
Mean		3.98
soleirolii		
PI 537242	Algeria	1.50
PI 537243	Australia	1.50
Mean		1.50
tenoreana		
PI 499161	Italy	1.25
truncatula		
PI 292436	Israel	2.25
PI 384648	Morocco	3.80
PI 566886	Italy	2.80
PI 566887	Greece	4.80
PI 566888	Australia	4.00
PI 566889	Turkey	4.60
PI 566890	Greece	3.60
PI 566891	Czechoslovakia	4.20
PI 566892	Spain	3.60
Mean		3.74
turbinata		
PI 441943	Syria	2.50
PI 535555	Tunisia	2.33
PI 566893	Lebanon	2.25
PI 566894	Turkey	2.25
PI 566895	Czechoslovakia	4.25
Mean		2.72
sativa[c]		
Ramsey (resistant)	USA	3.60
Ranger (susceptible)	USA	4.20

RESULTS

All 19 isolates of *P. medicaginis* produced symptoms of spring black stem and leaf spot on alfalfa cv. Ranger (Table 1). The isolates differed significantly ($P \leq 0.05$) in the ability to produce symptoms, but the majority produced a moderate level of disease. On a 0-to-5 scale, the range of ratings for symptoms was 2.4 to 4.7. The experiment was repeated and the results were similar, with each isolate receiving ratings within 1.2 points of the first rating. *P. medicaginis* isolate P1798 (T-431), with a virulence rating of 4.0 on Ranger, was selected for evaluations of lines in the annual *Medicago* spp. core collection.

Most of the accessions in the annual *Medicago* spp. core collection were susceptible to spring black stem and leaf spot (Table 2). Species with highly susceptible accessions included *M. arabica*, *M. lanigera*, *M. minima*, *M. rotata*, *M. rugosa*, *M. sauvagei*, and *M. scutellata*. Ten species whose accessions exhibited high resistance included *M. constricta*, *M. doliata*, *M. heyniana*, *M. laciniata*, *M. lesinsii*, *M. murex*, *M. orbicularis*, *M. praecox*, *M. soleirolii*, and *M. tenoreana*. Accessions of *M. murex* exhibited the highest level of resistance, with a mean disease rating of 1.1. Several accessions within *M. laciniata* and *M. lupulina* were highly resistant, with ratings of 0.0.

The degree of susceptibility found among accessions within species was variable. Within one of the more susceptible species, *M. polymorpha*, ratings ranged from 0.67 (PI 566873) to 4.29 (PI 566883). Most of the *M. truncatula* accessions were significantly more susceptible than accessions of other species. Disease ratings of the check cultivars of *M. sativa* ranged from 3.5 to 4.5 in Ranger and 3.0 to 4.0 in Ramsey.

DISCUSSION

The wide range of reactions expressed within and among accessions suggests that the medics harbor diverse sources of resistance to spring black stem and leaf spot. The significant range in susceptibility to *P. medicaginis* within accessions was not unexpected. Our results are consistent with results from field evaluations of annual *Medicago* spp. in South Africa (2). In particular, with few exceptions, their results revealed that the cultivars of *M. truncatula* and *M. polymorpha* that were tested were highly susceptible but included a range of disease reactions. In South African field studies, among 42 lines tested from five species, *M. murex* was found to have the most resistance (2). This species also was one of the most resistant in our tests with *P. medicaginis*, and also was reported to be highly resistant to anthracnose disease caused by *Colletotrichum trifolii* (26).

Only one highly virulent isolate of *P. medicaginis* was used to evaluate the resistance in the study reported here, and reactions to other strains of the pathogen may

vary. The strains evaluated for virulence in this study ranged in disease ratings from 2.4 to 4.7 on cv. Ranger (Table 1). Characterization of the population structure of *P. medicaginis* and related species has not yet been elucidated, and the degree of pathogenic and genetic variation that exists within alfalfa-infecting isolates from different geographical regions is unknown. These components will be important for understanding the biology of the fungus and for the development of effective methods to control disease. According to Salter and Leath, approximately 75 to 80% of the plants of cv. Ramsey and 97% of cv. Ranger were expected to be susceptible to the disease, which they described as moderately resistant and susceptible, respectively (30). In our test, Ramsey was not significantly different from Ranger. The selection of a highly virulent isolate, in conjunction with experimental conditions highly conducive to severe disease development, resulted in severe disease symptoms and ratings on Ranger as well as Ramsey (Table 2).

Accessions and cultivars of both annual and perennial *Medicago* spp. exhibit a wide range of disease reactions to diseases, including *P. medicaginis*, *Erysiphe pisi*, and *C. trifolii* (25,26,28,36). *Phoma* and *Colletotrichum* resistance are inherited characters in alfalfa (14,28); therefore, disease resistance traits could be utilized in a medic breeding program to increase the level of resistance to anthracnose and spring black stem and leaf spot available in commercial cultivars. It would be interesting to screen the world annual *Medicago* spp. core collection (31) located in Adelaide, South Australia, Australia (South Australian Research and Development Institute, Genetic Resources Collection) for resistance to these diseases. The Australian collection is composed of over 24,000 accessions and the core collection contains 1,705 accessions.

Resistance to major diseases in alfalfa increases quality, yield, and adaptability. Traditional methods of breeding alfalfa for resistance are limited by the autotetraploid nature of alfalfa and the severe effects of inbreeding depression. Annual *Medicago* spp. may be a source of resistance via new or different toxic secondary metabolites that suppress the pathogen and disease. An advantage to using annual medics is that they are simpler genetically to study and manipulate than perennial alfalfa. Resistance is the most desirable type of control for alfalfa pathogens because of the large acreage grown and the perennial character of the crop. Resistance to diseases caused by highly pathogenic foliar pathogens in alfalfa currently is needed, and new sources of disease-resistance genes should result from the identification of disease resistant accessions of annual *Medicago* spp. germ plasm. Such genes may facilitate the development of genetically engineered plants with broad spectrum disease resistance.

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